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The "form" makes the difference: unrevealing the differential impact of liquid and solid fructose in mice metabolic signatures by comprehensive two-dimensional gas chromatography

Chiara Cordero,[1*]Erica Liberto[1], Fausto Chiazza[1], Raffaella Mastrocola[2], Stephen E. Reichenbach[3], Carlo Bicchi[1] and Massimo Collino[1]

1: Dept. of Drug Science and Technology, University of Turin, Turin, Italy
2: Dept. of Clinical and Biological Sciences, University of Turin, Turin, Italy
3: Dept. of Computer Science and Engineering, University of Nebraska, Lincoln, NE, United States

Introduction:

Recent findings demonstrates that liquid and solid high-sugar diets differentially impact on feeding behaviour, hormone expression and intestinal sugar transporters [1]. This evidence suggests that not only the type (e.g. fructose vs. glucose), but also the form (liquid vs. solid) of sugars may affect the development of metabolic impairments. Reducing sugars have in fact differential reactivity in complex food systems thereby a similar behaviour is expected *in vivo*. In this challenging context, investigation approaches matching the high chemical dimensionality of thermally treated foods [2], can be of help to exploit and accurately track quali-quantitative changes in metabolic signatures of animal models subjected to high-fructose diets. Comprehensive two-dimensional gas chromatography coupled with mass spectrometry (GC×GC-MS) has been adopted in combination with advanced fingerprinting based on pattern recognition to understand, at the molecular level, the differential impact of fructose administrated in different formulations.

Methods:

Urine samples are taken from mice fed with normal or fructose enriched diets provided either in aqueous solution or in solid form and analyzed at three stages of the dietary intervention (1, 6, and 12 weeks). Automated *U*ntargeted and *T*argeted *fingerprinting* [3] for 2D data elaboration is adopted for the most inclusive data mining of GC×GC patterns. The *UT fingerprinting* strategy performs a fully automated peak-region features fingerprinting and combines results from pre-targeted compounds and unknowns across the sample-set. The most informative analytes, with statistically relevant differences between sample groups, are obtained by unsupervised multivariate analysis (MVA) and cross-validated by multi-factor analysis (MFA) with external standard quantitation by GC-MS.

Results:

Results indicate coherent clustering of mice urine signatures according to dietary manipulation clearly influenced by the administration form of fructose and its chemical reactivity in vivo. Notably, the metabolite fingerprints of mice fed with liquid fructose exhibited greater derangement in fructose, glucose, citric, pyruvic, malic, malonic, gluconic, cis-aconitic, succinic and 2-keto glutaric acids, glycine acyl derivatives (N-carboxy glycine, N-butyrylglycine, N-isovaleroylglycine, N-phenylacetylglycine), and hippuric acid. Untargeted fingerprinting indicates some analytes, not *a priori* pre-targeted, which provide additional insights: N-acetyl glucosamine, N-acetyl glutamine, malonyl glycine, methyl malonyl glycine, and glutaric acid. Visual features fingerprinting is used to track individual variations during experiments, thereby extending the panorama of possible data elaboration tools.

Conclusions:

Experimental data lead to the identification of different urine metabolite fingerprints recorded for the two diet groups. These findings are important since they confirm and further extend on-going observations on the differential toxicological impacts of solid *vs.* liquid fructose supplementation as a function of its bioavailability and distribution and its *in vivo* reactivity. Specifically, urine samples from mice fed by liquid formulations are clearly differentiated from the others, suggesting more significant metabolic alterations. The investigation approach based on advanced fingerprinting by GC×GC-MS and pattern recognition confirms its suitability to exploit samples where multiple chemical dimensions are

represented. A better understanding of complex biological phenomena is achieved when the investigation is driven down-to the molecular level and through known reaction pathways.

References

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